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THE EFFECT OF ENVIRONMENTAL FACTORS ON THE GROWTH OF A HALOPHYLIC SPECIES OF ALGAE

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True obligate halophylic algae will only grow well at elevated salt concentrations. These species have adapted very well to a severe environmental situation. An important question to ask about these halophytes is how they are able to survive such high salt concentrations, and what is the effect of various ecological parameters on their growth rates. The purpose of this study was to define the environmental factors limiting the growth of this species, and to see how these factors could be manipulated in order to produce high yields of cells for physiological and biochemical investigations.

Smith (1950) reported that the genus *Dunaliella* is worldwide in distribution and is almost invariably present in salterns and brine lakes. Butcher (1959) included 12 species in this genus, and stated they occur in diverse habitats including any situation from almost fresh water to salt brine. Many studies have been carried out on a variety of halophytes of the genus *Dunaliella* and *Chlamydomonas*. These studies have included, to name a few areas, culture methods and ecological parameters (Kirkpatrick, 1934; McLachlan, 1960; Yamada and Okamoto, 1961), sodium requirement (McLachlan, 1960), reproduction (Latorella, 1971), salt tolerance and osmoregulation (Baas-Becking, 1931; Marrè and Servettaz, 1959; Johnson, Johnson, MacElroy, Speer and Bruff, 1968; Okamoto and Suzuki, 1964; Yamamoto, 1967; Yamamoto and Okamoto, 1967, 1968; Wegmann, 1971), membrane characteristics (Jokela, 1969), photosynthesis (Ben-Amotz and Avron, 1972; Loeblich, 1972), and cell synchronization (Wegmann and Metzner, 1971).

Most algologists have tried to regulate the NaCl concentration of the culture media but have paid little attention to other parameters besides recognizing a minimal need and trying to satisfy this need in order to make their system work. Few definitive studies have been done concerning environmental regulation of growth of halophylic algae. High cell densities could be obtained but considerable time was required because of the slow growth rate. Usually one environmental factor or another was not considered, resulting in slow growth. The conditions that must be considered in order to regulate a population are both physical and chemical, and include the following: (1) culture media including chemical elements and their concentrations; (2) source and amount of carbon; (3) illumination; (4) temperature; (5) pH; and (6) salt concentration. Each of the above factors will be considered in the following experiments.

Materials and Methods

A series of collections were made from the brine of the Great Salt Lake, Utah, U.S.A. After several unsuccessful attempts, unialgal cultures of an unidentified

species of algae were obtained. According to H. C. Bold (Botany Dept., University of Texas, Austin, personal communication) and E. A. George (The Culture Centre of Algae and Protozoa, Cambridge, England, personal communication) the alga was an undescribed species of *Dunaliella*. These cultures were sustained in a medium containing major elements as described by Dyer and Gafford (1961) and minor elements in agreement with the work of Arnon (1938), plus 21% (w/v) NaCl. The *Dunaliella* cultures grew well once established, as did a number of halophylic bacteria. The medium was changed so that it was composed of the salts at the concentrations listed in Table I, resulting in better algal growth and a considerable reduction in the number of bacteria.

The next step in the isolation consisted of obtaining an axenic culture. This was accomplished by using the method of Wiedeman, Walne, and Trainor (1964) which consisted of washing the cells a number of times with sterile culture media containing a dilute detergent, spraying a dilute sample of algae on agar plates, and then picking and testing colonies for contamination. This method was very reliable.

TABLE I

Culture media

Salt	Concentration	Salt	Concentration
NaCl	175.329 g/l	$MnCl_2 \cdot 4H_2O$	1.81 mg/l
$Ca(NO_3)_2 \cdot 4H_2O$	0.662 g/l	$ZnCl_2$	0.11 mg/l
KH_2PO_4	0.313 g/l	$CuCl_2 \cdot 2H_2O$	0.050 mg/l
$MgSO_4$	0.277 g/l	$Na_2MoO_4 \cdot 2H_2O$	0.025 mg/l
Fe-EDTA	5.00 mg/l	$CoNO_3 \cdot 6H_2O$	0.050 mg/l
H_3BO_4	2.86 mg/l		

Stock cultures were kept on 1.5% plain agar (Bacto-Agar, Difco) which contained mineral elements as indicated in Table I and 17.5% (w/v) NaCl. Liquid cultures were grown in the same medium less agar. There were no vitamin supplements added to the medium and none are required, thus this species is a complete autotroph. Algal colonies were difficult to start on solid media, probably due to fragility of the cells because members of this genus do not have a cell wall. The cells cultured quite easily in liquid providing they were not exposed to harsh conditions during the transfer.

The alga cultures obtained were grown in mineral culture media aseptically and under a variety of conditions to determine their optimal growth response. Culture tubes 25 × 260 mm were used to raise the alga. The tubes were suspended from a ⅜″ plexiglass cover into a water bath. The water bath was thermo-regulated between 5° and 40° C ± 0.5° C depending on the desired condition by balancing the flow of cold tap water (flowing through a copper coil) with a heating element connected to a thermostat. Lower temperatures were obtained by using a recycling refrigeration unit.

Compressed air and CO_2 were added to the culture tubes at a rate of 2.18 ml/min/ml culture medium at concentrations from 0.0–4.4% CO_2 (v/v) at 8 psi. The dry mixture of gas was filtered first through activated charcoal (40 mesh), second through sterile cotton, before it was saturated with water, and finally de-

livered to the culture through a 0.034" diameter polyethylene tubing which was inserted into the culture through a plug. The light source at the lower intensities consisted of two 40-watt cool white fluorescent lamps; at higher intensities the source included four high intensity white fluorescent lamps. Light intensities were measured with a GE Light Meter or a Weston Illumination Meter Model 75. The suspension were maintained on a 20–4 hour light-dark period (to maintain high growth rates). Sodium chloride in the media was varied between 0.0 and 35% (w/v) as required.

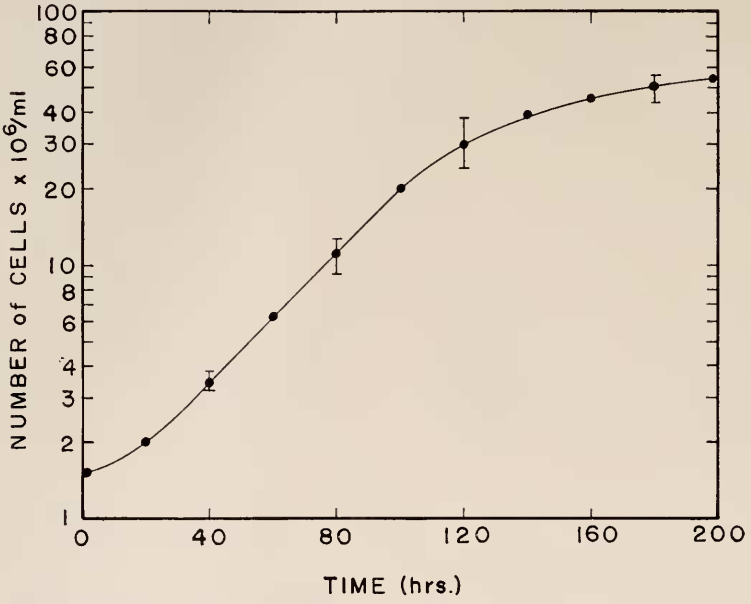

FIGURE 1. Growth curve for *Dunaliella* sp.; temperature 30° C, 21.0% NaCl, 2% CO_2, initial pH 5.5, light intensity 5.35 Klux (1 Klux = 93.4 ft-c), and a 20–4 hr light-dark cycle. Each point represents the mean of at least five determinations and the vertical bars represent ± one standard deviation.

The pH of the culture media was adjusted to 5.5 with 0.1 M NaOH and was then measured during the entire growth cycle (the pH could not be maintained more neutral because of the high CO_2 levels). A Beckman Expandomatic pH meter was used for pH determination. No Na^+ correction was needed in the range of pH's measured (Beckman Instruments, Inc., 1950). A specific buffer was not used in the media.

Cell populations were measured at various time intervals using a model "F" Coulter Counter with a 100 μ aperture (Coulter Electronics, Inc, 1967). The data were then plotted and from the resultant growth curve (Fig. 1) the specific growth rate (k) was calculated from which the doubling time (t_2) was determined (Myers, 1962). Next the t_2's were plotted *versus* the magnitude of the variable being investigated. The Coulter Counter worked very well in determining cell populations,

but it was time consuming; consequently, a Beckman Model "B" spectrophotometer was calibrated at 750 mm using known cell dilutions. A standard curve was plotted and then the Model "B" was employed to make turbidometric measurements of the algal populations.

Carbon dioxide concentrations present in the ambient air were measured with a Beckman Model 215 A IR Gas Analyzer using standard CO_2 mixtures for comparison. A zero CO_2 concentration was obtained by passing the ambient air through three 2-liter flasks containing 1200 ml of 20% (w/v) KOH. Concentrations of CO_2 above ambient were obtained by mixing compressed CO_2 at a given temperature, pressure and flow rate with ambient air at the same temperature, pressure and a flow rate calculated to give the desired final concentration.

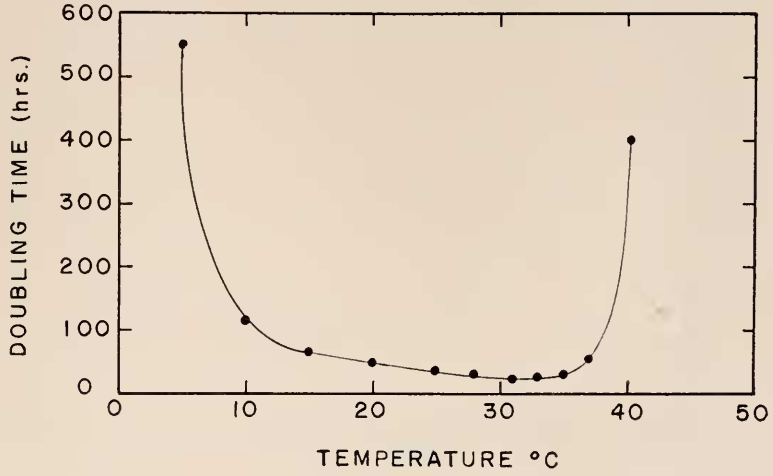

FIGURE 2. The effect of temperature on the doubling time (t_d). Growth conditions were: 21.0% (w/v) NaCl, 2% CO_2, initial pH 5.5, light intensity 5.35 Klux, and a 20–4 hr light-dark cycle. Each point represents the mean of at least five determinations.

For experiments on the sodium requirements of the algae, the cells were grown in 19.2% (w/v) or 28.0% (w/v) NaCl, and then centrifuged and resuspended in a small volume of the growth media. The cell population was determined and an aliquot of media containing 1×10^6 cells was placed in complete culture media with altered K^+/Na^+ ratios varying from 10^3 to 10^{-3}. Environmental conditions were optimal for growth. The doubling times were determined and plotted against the log of the K^+/Na^+ ratio.

All of the experiments were begun by inoculating five culture tubes with a large enough volume of inoculum so that the initial concentration of cells was 1×10^6/ml. The growth of the cultures was monitored spectrophotometrically and the data recorded and plotted. The doubling times were determined from the growth curves and finally plotted. In some cases, standard deviations were calculated and are shown in the appropriate figures.

Results

Optimum temperature for growth was determined for the cells in 19.2% (w/v) NaCl medium at pH 5.5, 2% CO_2, 5.35 Klux and over a temperature range from 5° C–40° C (Fig. 2). Cultures were inoculated with 1×10^6 cells/ml. Cell populations were measured, growth rates were calculated, at least five replications were used, and the averages of the t_2's were then plotted *versus* temperature. Maximum growth took place at 32° C with the $t_2 = 23.8$ hrs. At 5° C and 40° C, the t_2's were 550 hrs and 415 hrs, respectively. At the lower temperature the cells were

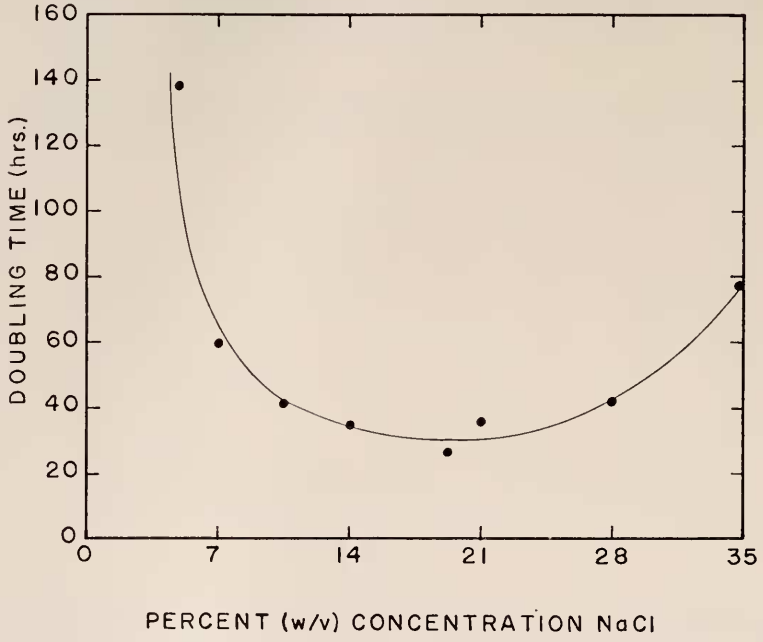

Figure 3. The effect of the NaCl concentration on the doubling time. Growth conditions were: 30° C, 2% CO_2 initial pH 5.5, 5.35 Klux, and a 20–4 hr light-dark cycle. Each point represents the mean of at least five determinations.

normal and active, but had a greatly decelerated growth rate. At the higher temperature the cells became distorted and abnormally large, probably due to damage to cell enzymes, particularly those involved with the osmoregulation system.

Cells were grown at 30° C, pH 5.5, 2% CO_2, 5.35 Klux light intensity and in culture medium ranging in concentration from 0.0 to 35% (w/v) NaCl in order to define the optimum NaCl concentration for growth (Fig. 3). Inoculation of cultures and the number of experiments were as previously described except when the NaCl concentration was changed. If the NaCl concentration was changed, it was accomplished by dilution with distilled water, or by addition of culture media with a higher NaCl concentration, and the change was not more than 3.5% at any one time. Changes were completed in a six-hour period, but the cells were not transferred to begin a new growth experiment for another four to six hours. When

the cells were transferred it was noted that the lag time was short, comparable to those cultures not transferred, indicating no damage to the cells due to change in osmotic environment. Maximum growth occurred in 2.75 M or 19.2% (w/v) NaCl with a t_2 of 30 hrs. The t_2's were infinity for 3.15% (w/v) NaCl and 77 hrs for 35% NaCl. At NaCl concentration lower than 3.15% the cells did not grow, thus a death rate occurred rather than a growth rate, and this is not plotted in Figure 3.

Below 7% (w/v) NaCl the doubling time was very hard to determine because of "frothing" of the cultures. The cells clumped together and would not remain in suspension; consequently, there were very few measurements in this range and a high degree of variability.

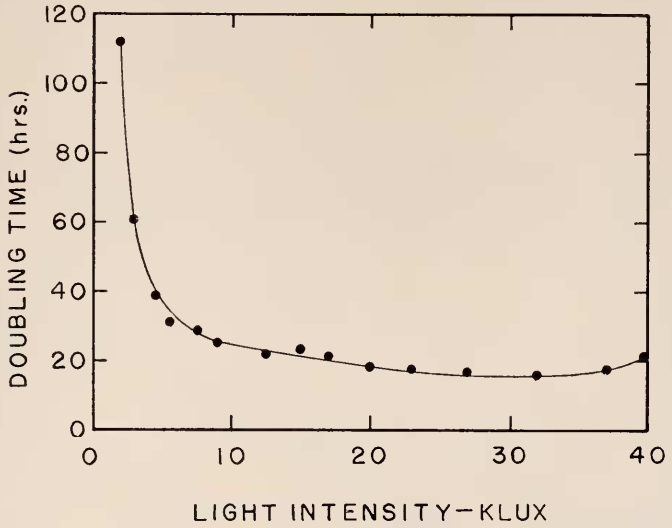

FIGURE 4. The effect of the light intensity on the doubling time. Growth conditions were: 30° C, 2% CO_2, initial pH 5.5, NaCl 19.2% (w/v), and a 20–4 hr light-dark cycle. Each point represents the mean of at least five determinations.

In order to measure the optimum light intensity the k was determined for the algae grown in 19.2% (w/v) NaCl culture medium at 30° C, pH 5.5, 2% CO_2, and over a range of light intensities from 2.12 Klux to 40.6 Klux (Fig. 4). The inoculation of cultures and the number of experiments were as described previously. Maximum growth took place between 25 and 35 Klux, and at this intensity $t_2 =$ 16 hrs. The doubling time approached infinity at a light intensity less than 1 Klux. A decrease in the growth rate also occurred at high light intensities (35–40 Klux), probably due to pigment bleaching or photo-oxidation.

The t_2 for the algae was also investigated at CO_2 concentrations varied from 0.0% to 4.4% (v/v) (Fig. 5), and the optimum concentration was determined. The light intensity was held constant at 26.8 Klux (technical difficulties caused the utilization of a slightly lower than optimum intensity), the temperature at 30° C, NaCl concentration at 19.2% (w/v) and the pH, inoculation, and number of replica-

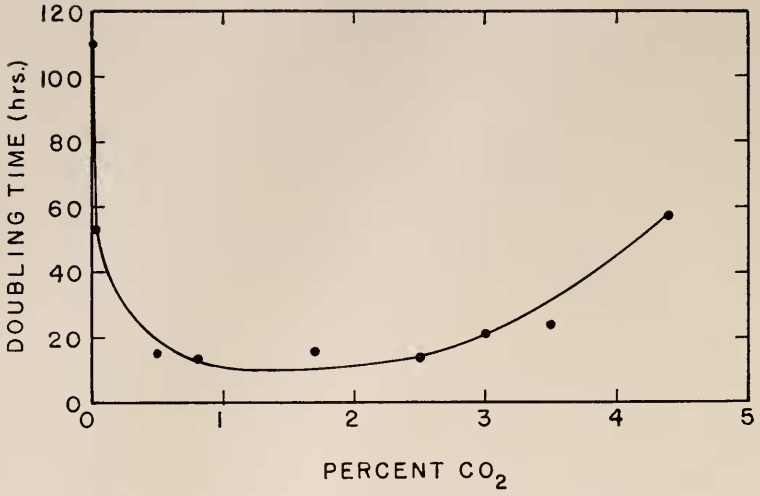

FIGURE 5. The effect of the CO_2 concentration on the doubling time. Growth conditions were: 30° C, intial pH 5.5, light intensity 26.8 Klux, NaCl 19.2% (w/v), and a 20–4 hr light-dark cycle. Each point represents the mean of at least five determinations.

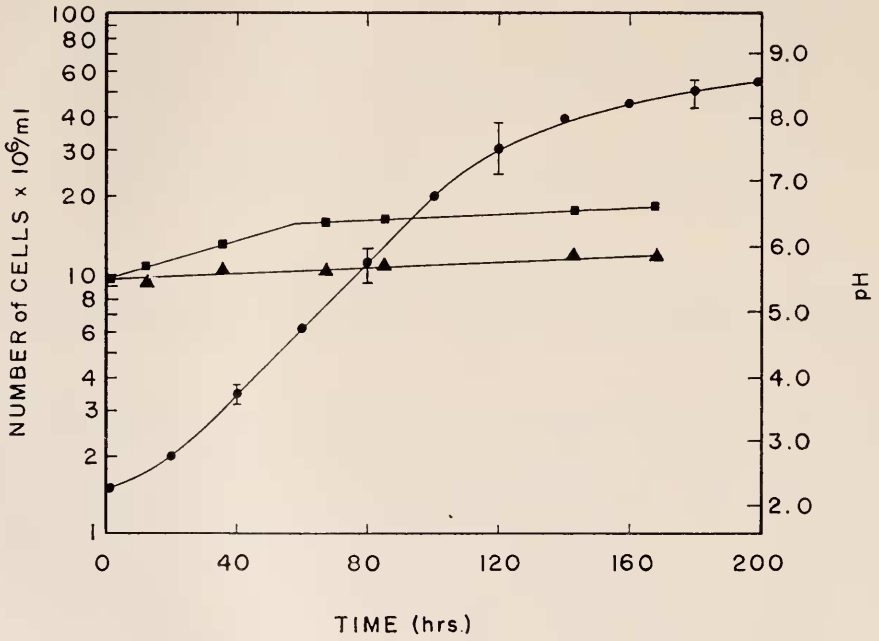

FIGURE 6. The effect of algal growth on the pH of the growth media, and the effect of the pH on the growth curve. Growth conditions were as follows: 30° C, 21.0% (w/v) NaCl, 2.0% CO_2, 5.35 Klux. Cells/ml (solid circles); pH of medium (solid squares). Each point represents the mean of at least five determinations, and the vertical bars represent ± one standard deviation; aerated uninoculated media (solid triangles).

tions were maintained as previously noted. Under these conditions maximum growth took place between 1 and 2% CO_2, with $t_2 = 10$ hrs.

The pH of the growing culture was determined at the same time as the growth rates were measured to see if any change in the pH of the culture occurred and to determine if the change in pH caused a change in the growth rate. The results of the experiment are plotted in Figure 6. The number of cells/ml is plotted on the left-hand vertical axis and the pH on the right-hand vertical axis, and both are plotted against time. The uninoculated aerated culture medium showed a small pH change (Fig. 6). The culture medium inoculated with 1.5×10^6 cells/ml increased in pH at the rate of 1.56×10^{-2} pH units/hr up to 60 hours and a pH of 6.4. After 60 hours, the pH rose very slowly 2.2×10^{-3} pH units/hr up to 170 hours and

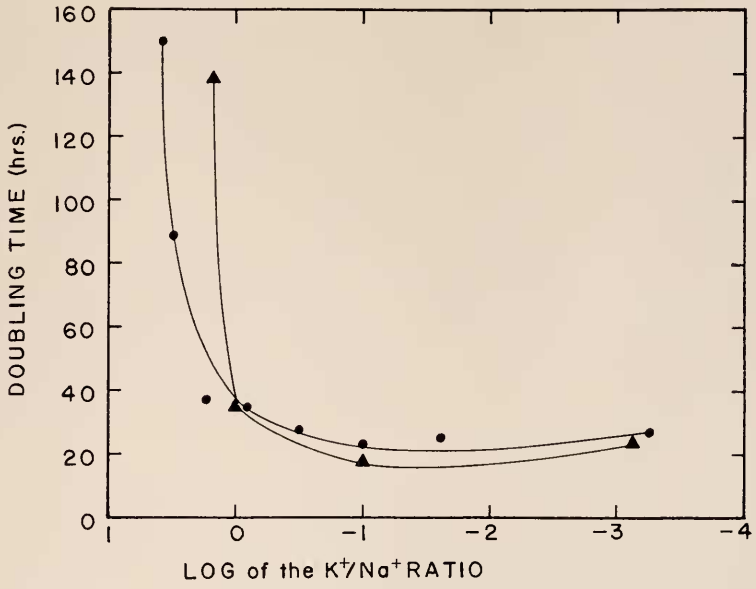

FIGURE 7. The effect of the K^+/Na^+ ratio at 19.2% (solid triangles) and 28.0% (solid circles) on the doubling time. Each point represents the mean of at least five determinations.

a pH of 6.5. Figure 6 shows the growth curve and the pH curve plotted against time. One can see that the pH over the range 5.5–6.5 has no effect on the log phase of the growth curve. The same figure shows that the pH is altered rather markedly by the presence of the algae.

Figure 7 shows the effect of the K^+/Na^+ ratio on the doubling time. The optimum t_2 occurred between a K^+/Na^+ ratio of 0.1 and 0.001 and decreased at both lower and higher ratios. At the K^+/Na^+ ratio of 1.0 the k decreased very rapidly The same phenomenon occurred in both 19.2% and 28.0% salt concentrations.

DISCUSSION

The halophylic green algae are remarkably similar to the marine and freshwater algae in their requirements and growth response, except for their high requirement for an osmotically active substance, usually NaCl.

Innumerable formulas exist for media, variously selected by different laboratories (Society of Protozoologists, 1958). In the present study, most of the elements listed by Nicholas (1963) were used in suitable concentrations and the NaCl concentration was adjusted to that at which near optimal growth took place. Other authors (Gibor, 1956; Provasoli, McLaughlin and Droop, 1957; Yamada and Okamoto, 1961; Johnson *et al.* 1968) have used similar techniques.

All halophylic algae investigated have been grown at temperatures between 14° C and 30° C (Gibor, 1956; McLachlan, 1960; Yamada and Okamoto, 1961; Johnson *et al.*, 1968). From the above data and the data collected in the present study, it appears that the halophytes are mesothermal species. The growth rates of the undescribed *Dunaliella* were decreased at temperatures above and below 32° C, but 32° C was optimum for this species.

Light intensities that have been used to culture algal halophytes range from 1–22 Klux (Gibor, 1956; McLachlan, 1960; Yamara and Okamoto, 1961; Johnson *et al.*, 1968; Wegmann, 1971; Loeblich, 1972). At all but the highest intensities growth may actually be limited by low light intensity, but a low CO_2 concentration would be limiting at a higher intensity. In the present study, the t_2 was very low at light intensities of 1–5 Klux, optimum light intensity being about 25–35 Klux, but these measurements were made at high CO_2 concentrations. At the higher light intensity and higher CO_2 concentration, the t_2 was 16 hrs, which was a shorter doubling time than reported for any other halophlic algae.

The pH of the Great Salt Lake was reported between 7.4–8.4 (Flowers and Evans, 1966), depending on the location and the ions present. The marine environment also falls within this same range (Sillen, 1967). The pH of all reported growth experiments with halophylic species of *Chlamydomonas* and *Dunaliella* have been carried out between pH 7 and 9 (Gibor, 1956; Provasoli, McLaughlin and Droop, 1957; McLachlan, 1960; Yamada and Okamoto, 1961; Johnson *et al.*, 1968; Wegmann, 1971; Loeblich, 1972).

The species of *Dunaliella* used in this study has a wide range of pH tolerance. The growth conditions suggest the cells are not affected by broad changes in pH (5.5–6.5). Other experiments (to be reported elsewhere) show that there is very little change in the photosynthetic or respiratory rate from 4.5–8.5 pH units.

Provasoli, McLaughlin and Droop (1957) suggest 4% CO_2 enrichment for growing marine algae. For many species of algae carbon as glucose or acetate may be provided rather than CO_2 (Myers, 1962). However, some organisms are obligate photo-autotrophs and cannot utilize organic carbon from the culture medium.

All of the halophylic species of green algae investigated seem to be completely autotrophic (Gibor, 1956; McLachlan, 1960; Yamada and Okamoto, 1961; Johnson *et al.*, 1968; Wegmann, 1971; Loeblich, 1972). In all of the above cases, if CO_2 enrichment had been used coupled with higher light intensities (previous discussion), the t_2 would probably have decreased, indicating a shorter doubling time. The species reported here responded very favorably to increased CO_2 concentration. A high degree of stimulation occurred with a relatively small change in CO_2 concentration. An optimum growth rate occurred at 1–2% CO_2 at a given flow rate and pressure, but these cells were grown at elevated light intensities. A lowered pH at high CO_2 levels could have caused the increase in doubling time, but this information is not available at the present time.

Salt concentrations from 0–35% NaCl have been used to culture the brine algae (McLachlan, 1960; Yamada and Okamoto, 1961; Johnson *et al.*, 1968; Wegmann, 1971; Wegmann and Metzner, 1971; Loeblich, 1972). Optimum growth occurred at 19.2% NaCl for the unidentified *Dunaliella* reported in this paper, thus it is physiologically separate from other members of the genus. The t_2 was maximum at this concentration but there was a wide range of tolerance. The greatest effect of NaCl concentration occurs at the low salinity range, and the t_2 increases rapidly below 7.0% NaCl. The osmoregulation system may be overloaded at the low concentration of NaCl; or there may be a leakage of the plasma membrane or an increase in secretion of metabolic constituents into the media. There is some evidence of the latter effect shown by an apparent increase in viscosity of the growth medium.

Cells growing in environments containing a high concentration of Na^+ could have a definite Na^+ requirement. McLachlan (1960) showed a Na^+ requirement for *D. tertiolecta*, but generally it is very difficult to prove a definite requirement of a metal ion for a given organism (Arnon, 1938), especially for such a common metal ion as Na^+. This experiment (Fig. 7) does not show an absolute Na^+ requirement, but it shows a definite dependence on the amount of Na^+ in the medium and suggests that the ion cannot be substituted for, at least by K^+ at the concentrations used in these experiments. There is a possibility that another osmotic agent could be used to substitute for the Na^+ or both the Na^+ and the Cl^-. A compound like glycerol, carbowax (polyethylene glycol), sugar, or sugar alcohol, or even another alkali or alkali earth salt could be used. These experiments have not been carried out to date because of two problems: one being toxicity of the substitute compound; and the other being low solubility.

With cells grown in a 2.75 M salt media, the optimum t_2 occurred at a K^+/Na^+ ratio of 0.01, but at a 1/1 K^+/Na^+ ratio the t_2 decreased very rapidly. As the K^+ concentration increased and the Na^+ concentration decreased, the specific growth rate decreased. This suggested that at least the K^+ to Na^+ ratio was very important; and once a certain level is reached, the cells do not function properly. Possibly the internal K^+ concentration was at such a high level it inhibited certain enzymatic reactions by substituting for Na^+ at the active site. Sorger, Ford and Evans (1965) showed a definite requirement of some enzymes of mesophytes for Na^+ or K^+. They reported that some substitution of ions could occur, but the activity was reduced. Another possibility could be that K^+ was actively absorbed until a much higher internal K^+ concentration occurred; and if this happened, the high ionic strength of the K^+ could precipitate some enzymes, or change their charge distribution and cause them to be inactive or to have a lower activity. The cause of the decrease in the specific growth rate is not known, but the experiment showed that the cells would not grow in a high K^+ medium; however, they would grow in a high Na^+ medium, suggesting a Na^+ requirement. At the same time, there was a slight increase in the t_2 at low K^+ concentrations suggesting a K^+ requirement. The cells in these experiments were not killed by osmotic shock because changes in osmodicum occurred over an eight to twelve-hour period.

The same experiment was carried out at a 4.0 M concentration, and the results were very similar. The decrease in growth rate at a higher salt concentration was

expected and occurred in other experiments. This decrease could have been due to a higher internal NaCl concentration, an osmotic response, and/or a reduction in enzyme activity (Johnson *et al.,* 1968), or it could have been due to a lower P/R ratio, resulting from a greater energy expenditure for osmoregulation (McLusky, 1969.) The optimum doubling occurred at the same K^+/Na^+ ratio and a very steep decline occurred at a K^+/Na^+ ratio of 1.0. The doubling time increased to infinity at 0.1 M and beyond that point the specific growth constants were negative (death rates) and are not shown in Figure 7, but the magnitude of decrease was not as great as in the 2.75 M medium. One possibility to be considered is that even though the K^+/Na^+ ratio was the same, there were more Na^+ ions present at the higher molarity than there were in the 2.75 M media thus modifying the deleterious effect.

The optimum conditions for the growth of this species of *Dunaliella* as determined in these experiments are as follows: NaCl 19.2%, CO_2 1–2% delivered at a rate of 2.18 ml/min/ml culture solution, temperature 32.0° C, light intensity 25–35 Klux, and a pH range from 5.8–6.5. Under these conditions the doubling time (t_2) was 10 hours. The optimum conditions described here ignore any interaction between factors tested.

The specific growth rate and the doubling time for optimal growth can be compared directly with freshwater, marine or other brine algae. Specific growth rates and t_2's reported were not greatly different from those reported for other species of algae grown under optimum conditions (McLachlan, 1960; Yamada and Okamoto, 1961; Talling, 1962; Wilson and Lords, 1965; Fogg, 1965; and Loeblich, 1972).

Of all the factors tested the one most limiting was CO_2 concentration (Fig. 5). A very slight increase in CO_2 concentration over ambient caused a very marked change in the t_2 (Fig. 5). This dramatic increase in growth rate did not occur for any of the other variables tested, at least not in the "normal" physiological or ecological range, but at the limits drastic changes in doubling time occurred.

This species is not unusual because it grows and divides at a given rate, a rate at least equal to that of many freshwater and marine forms; but that it can grow and divide at this rate in a saturated NaCl medium, and that growth does not occur at all in media containing less than 3.15% NaCl.

One of the postulated reasons for a maximum growth rate at a high salt concentration for the algae is that the internal salt concentration is lower than that of the medium (Yamada and Okamoto, 1961; Johnson *et al.*, 1968). If this is the case, another active osmotic agent must be present, and evidence has been presented to show that glycerol may be this agent (Craigie and McLachlan, 1964; Jokela, 1969; Wegmann, 1971). Next, one should ask why the cells do not grow well at salt concentrations higher or lower than optimum. This point remains to be investigated; but possibly the osmoregulation system is inhibited at higher and lower salt concentrations due to a breakdown of the control caused by a reduced energy supply (inhibited respiration or photosynthesis), decreased glycerol production or alterations of the Na^+/K^+ pumping or regulatory system which may lead to the rupturing of the cells, or reduced growth rates. There may be other factors involved, but they are not known at the present time.

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Summary

A halophylic species of *Dunaliella* was isolated from the Great Salt Lake, Utah, and established in axenic culture. A balanced culture media was developed containing major and minor elements as well as a sufficient concentration of an osmotic agent. The effects of various environmental factors on the growth of this species of algae were investigated and optimum growing conditions were delineated. Optimum conditions for growth of this species are as follows: (1) temperature 32° C; (2) NaCl 19.2% (w/v); (3) CO_2 1–2% at a rate of 2.2 ml/min/ml of culture media; (4) light intensity 25–35 Klux; and (5) pH 5.8–6.5. The K^+/Na^+ ratio should not be more than one, and better growth took place when this ratio was less than 0.1. The specific growth constant for this halophyte under the above conditions was 0.069 hrs^{-1}, which is equal to a doubling time of 10.0 hours.

LITERATURE CITED

Arnon, D. I., 1938. Microelements in culture-solution experiments with higher plants. *Amer. J. Bot.*, **25**: 322–325.

Baas-Becking, L. G. M., 1931. Salt effects on swarmers of *Dunaliella viridis* Teod. *J. Gen. Physiol.*, **14**: 765–779.

Beckman Instruments, Inc., 1950. Instructions for the use of Beckman glass electrodes made with general purpose glass. *Beckman Bull.*, **255-A**: 2.

Ben-Amotz, A., and M. Avron, 1972. Photosynthetic activities of the halophilic alga *Dunaliella parva*. *Plant Physiol.*, **49**: 240–243.

Butcher, R. W., 1959. An introductory account of the smaller algae of British coastal waters. *Fish. Invest. Minist. Agric. Fish. Food (Gr. Brit.) Series IV*, **Part 1**: 21–24.

Craigie, J. S., and J. McLachlan, 1964. Glycerol as a photosynthetic product in *Dunaliella tertiolecta* Butcher. *Can. J. Bot.* **42**: 777–778.

Coulter Electronics, Inc., 1967. *Coulter Counter Instructions and Service Manual for the Model "F"*. Coulter Electronics, Inc., Hialeah, Florida, 125 pp.

Dyer, D. L., and R. D. Gafford, 1961. Some characteristics of a thermophilic blue-green alga. *Science*, **134**: 616–617.

Flowers, S., and F. R. Evans, 1966. The flora and fauna of the Great Salt Lake region, Utah. Pages 367–393 in Hugo Boyko, Ed., *Salinity and Aridity; New Approaches to Old Problems*. W. Junk, The Hague.

Fogg, G. E., 1965. *Algal Cultures and Phytoplankton Ecology*. University of Wisconsin Press, Madison, 126 pp.

Gibor, A., 1956. The culture of brine algae. *Biol. Bull.* **111**: 223–229.

Johnson, M. K., E. J. Johnson, R. D. MacElroy, H. L. Speer and B. S. Bruff, 1968. Effects of salts on the halophilic alga *Dunaliella viridis*. *J. Bacteriol.*, **95**: 1461–1468.

Jokela, A. C. T., 1969. Outer membrane of *Dunaliella tertiolecta:* isolation and properties. *Dissertation, University of California, San Diego*, 123 pp.

Kirkpatrick, R., 1934. Life of the Great Salt Lake with special reference to the algae. *Unpublished Master's thesis, University of Utah*, 30 pp.

Latorella, A. H., 1971. Growth and salinity adaptation in the phytoflagellate, *Dunaliella tertiolecta*. *Dissertation, University of Maine*, 106 pp.

Loeblich, L. A., 1972. Studies on the brine flagellate *Dunaliella salina*. *Dissertation, University of California, San Diego*, 157 pp.

Marrè, E., and O. Servettaz, 1959. Sul meccanismo di adattamento a condizioni osmotiche estreme in *Dunaliella salina*. II. Rapporto fra concentrazioni del mezzo esterno e composizione del succo cellulare. *Atti Accad. Naz. Lincei Rend. Cl. Sci. Fis. Mat. Natur.*, **26**: 272–278.

McLachlan, J. 1960. The culture of *Dunaliella tertiolecta* Butcher—a euryhaline organism. *Can. J. Microbiol.* **6**: 367–379.

McLusky, D. S., 1969. The oxygen consumption of *Corophium volutator* in relation to salinity. *Comp. Biochem. Physiol.* **29**: 743–753.

Myers, J., 1962. Laboratory cultures. Pages 603–615 in R. A. Lewin, Ed., *Physiology and Biochemistry of Algae.* Academic Press, New York.

Nicholas, D. J. D., 1963. Inorganic nutrient nutrition of microorganisms. Pages 363–447 in F. C. Steward, Ed., *Plant Physiology; Vol. III; Inorganic Nutrition of Plants.* Academic Press, New York.

Okamoto, H., and Y. Suzuki, 1964. Intracellular concentration of ions in a halophilic strain of *Chlamydomonas.* I. Concentration of Na, K, and Cl in the cell. *Z. Allg. Mikrobiol.*, **4**: 350–357.

Provasoli, L., J. A. McLaughlin and M. R. Droop, 1957. The development of artificial media for marine algae. *Archiv. Mikrobiol.*, **25**: 392 428.

Sillen, L. G., 1967. The ocean as a chemical system. *Science*, **156**: 1189–1197.

Smith, G. M., 1950. *The Fresh-Water Algae of the United States.* [2nd ed.] McGraw-Hill, New York, 719 pp.

Society of Protozoologists, 1958. A catalogue of laboratory strains of free-living and parasitic protozoa. *J. Protozool.*, **5**: 1–38.

Sorger, G. J., R. E. Ford and H. J. Evans, 1965. Effects of univalent cations on the immunoelectrophoretic behavior of pyruvic kinase. *Proc. Nat. Acad. Sci.*, **54**: 1614–1621.

Talling, J. F., 1962. Freshwater algae. Pages 743–757 in R. A. Lewin, Ed., *Physiology and Biochemistry of Algae.* Academic Press, New York.

Wegmann, K., 1971. Osmotic regulation of photosynthetic glycerol production in *Dunaliella. Biochim. Biophys. Acta*, **234**: 317–323.

Wegmann, K., and H. Metzner, 1971. Synchronization of *Dunaliella* cultures. *Arch. Mikrobiol.* **78**: 360–367.

Wiedeman, V. E., P. L. Walne and F. R. Trainor, 1964. A new technique for obtaining axenic culture of algae. *Can. J. Bot.*, **42**: 958–959.

Wilson, D. G., and J. L. Lords, 1965. Growth characteristics of *Microcystis aeruginosa* cultured at elevated temperatures. *Proc. Utah Acad. Sci. Arts Lett.*, **42**: 159–160.

Yamada, H., and H. Okamoto, 1961. Isolation and pure culture of a halophilic strain of *Chlamydomonas. Z. Allg. Mikrobiol.*, **1**: 245–250.

Yamamoto, M., 1967. Osmotic regulation in a halophilic strain of *Chlamydomonas.* II. Effects of sulfhydryl reagents. *Z. Allg. Mikrobiol.*, **7**: 267–277.

Yamamoto, M., and H. Okamoto, 1967. Osmotic regulation in a halophilic *Chlamydomonas* cell. I. General feature of the response to the change in osmotic pressure. *Z. Allg. Mikrobiol.*, **7**: 143–150.

Yamamoto, M., and H. Okamoto, 1968. Osomotic regulation in a halophilic *Chlamydomonas* cell. III. Mg^{++} — and Ca^{++} — dependent adenosine-triphosphatase in microsome fraction. *Z. Allg. Mikrobiol.*, **8**: 71–84.